AD-A193 264 MUTAGENIC POTENTIAL OF NITROGUANIDINE IN THE AMES SALMONELLA/MAMMALIAN MI..(U) LETTERMAN ARMY INST OF RESEARCH PRESIDIO OF SAN FRANCISCO CA.. 1/1

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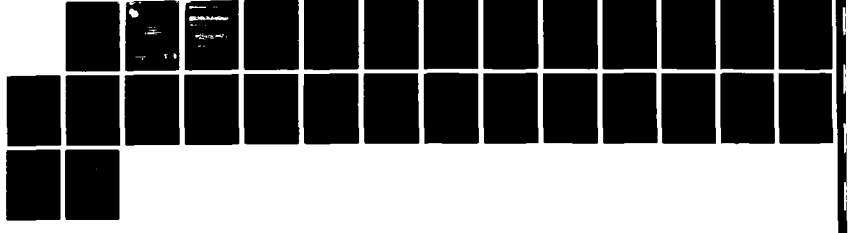

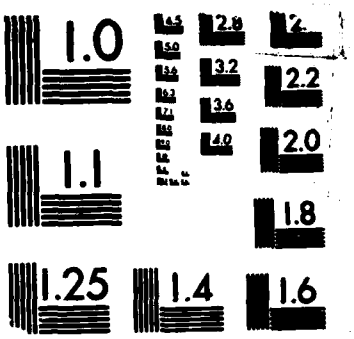

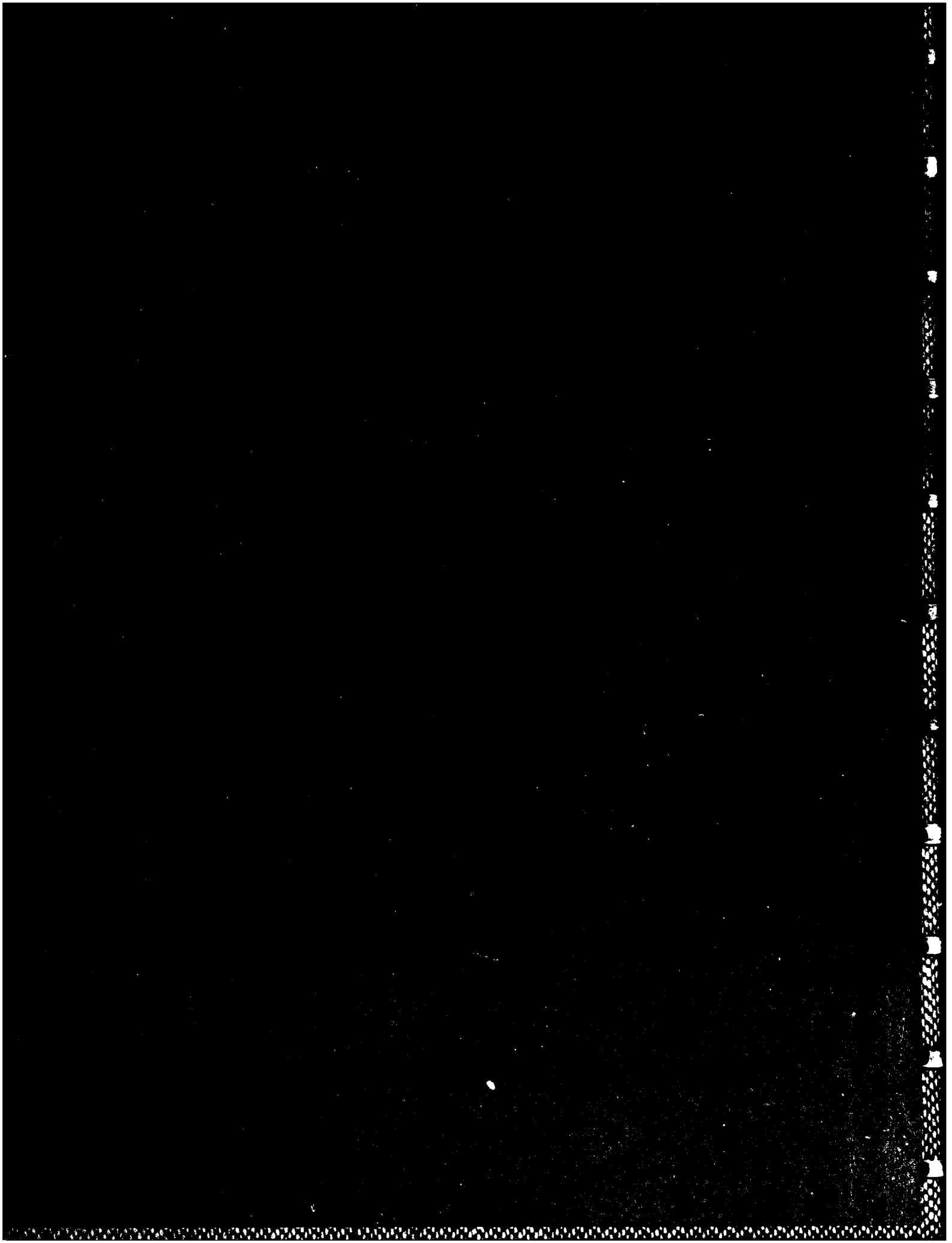

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Mutagenic potential of nitroguanidine in the Ames Salmonella/mammalian microsome mutagenicity test

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The mutagenic potential of NITROGUANIDINE was assessed by using the Ames Salmonella/Mammalian Microsome Mutagenicity Test. Tester strains TA97, TA98, TA100, TA102, TA1535, TA1537, and TA1538 were exposed to doses ranging from 2.8 mg/plate to 0.0875 mg/plate. The test compound was not mutagenic under conditions of this test.

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ABSTRACT

The mutagenic potential of NITROGUANIDINE was assessed by using the Ames Salmonella/Mammalian Microsome Mutagenicity Test. Tester strains TA97, TA98, TA100, TA102, TA1535, TA1537, and TA1538 were exposed to doses ranging from 2.8 mg/plate to 0.0875 mg/plate. The test compound was not mutagenic under conditions of this test.

Key Words: Mutagenicity, Genetic Toxicology, Ames Test, NITROGUANIDINE.

PREFACE

TYPE REPORT: Ames Test GLP Study Report

TESTING FACILITY: US Army Medical Research and Development Command
Letterman Army Institute of Research
Presidio of San Francisco, CA 94129-6800

SPONSOR: US Army Medical Research and Development Command
US Army Biomedical Research and Development Laboratory
Fort Detrick, Frederick, MD, 21701-5010
Project Officer: Gunda Reddy, PhD

PROJECT/WORK UNIT/APC: 3E162720A835/180/TLBO

GLP STUDY NUMBER: 86008

STUDY DIRECTOR: MAJ Don W. Korte Jr, PhD, MSC

PRINCIPAL INVESTIGATOR: Suzanne E. Sebastian, BA, SP4, USA

REPORT AND DATA MANAGEMENT: A copy of the final report, study protocol, retired SOP's, stability and purity data on the test compound, and an aliquot of the test compound will be retained in the LAIR Archives.

TEST SUBSTANCE: NITROGUANIDINE

INCLUSIVE STUDY DATES: 28-30 October 1986

OBJECTIVE: The objective of this study was to determine the mutagenic potential of NITROGUANIDINE (LAIR Code TP 36A) by using the Ames Salmonella/Mammalian Microsome Mutagenicity Test.

ACKNOWLEDGMENTS

MAJ John W. Harbell, PhD, MSC; SGT Lillie D. Witcher, BS; and SGT Gayle A. Orner, BS, provided research assistance.

SIGNATURES OF PRINCIPAL SCIENTISTS INVOLVED IN THE STUDY

We, the undersigned, declare that GLP Study 86008 was performed under our supervision, according to the procedures described herein, and that this report is an accurate record of the results obtained.

Don W. Korte Jr 16 FEB 88

DON W. KORTE Jr, PhD / Date
MAJ, MSC
Study Director

Suzanne E. Sebastian 12 February 88

SUZANNE E. SEBASTIAN, BA / DATE
SP4, USA
Principal Investigator

DEPARTMENT OF THE ARMY

LETTERMAN ARMY INSTITUTE OF RESEARCH
PRESIDIO OF SAN FRANCISCO, CALIFORNIA 94129-6800

REPLY TO
ATTENTION OF:

SGRD-ULZ-QA (70-1n) 23 February 1988

MEMORANDUM FOR RECORD

SUBJECT: Report of GLP Compliance for 86008, Tox Series 107

1. I hereby certify that the protocol was reviewed on 29 October 1986.

2. The report and raw data for this study were audited on 24 November 1987.

Carolyn M. Lewis
CAROLYN M. LEWIS
C, Quality Assurance

TABLE OF CONTENTS

Mutagenic Potential of NITROGUANIDINE - Sebastian and Korte

NITROGUANIDINE, a primary component of US Army triple-base propellants, is now produced in a Government-owned contractor-operated ammunition plant. The US Army Biomedical Research and Development Laboratory (USABRDL), as part of its charge to evaluate the environmental and health hazards of propellants generated by US Army munitions manufacturing facilities, conducted a review of the nitroguanidine data base and identified significant gaps in the toxicity data (1). The Division of Toxicology, LAIR, was tasked by USABRDL to develop a genetic and mammalian toxicity profile for nitroguanidine and related intermediates/by-products of its manufacture or environmental degradation products.

The Ames Salmonella/Mammalian Microsome Mutagenicity Test is a short-term screening test that utilizes histidine auxotrophic mutant strains of Salmonella typhimurium to detect compounds that are potentially mutagenic in mammals. A mammalian microsomal enzyme system is incorporated in the test to increase sensitivity by simulating in vivo metabolic activation of the test compound. The Ames test is an inexpensive yet highly predictive and reliable test for detecting mutagenic activity and thus carcinogenic potential (2).

This evaluation of NITROGUANIDINE utilizes a revision of the Ames Salmonella/Mammalian Microsome Mutagenicity Test (3). Two new tester strains, a frame-shift strain (TA97) and a strain carrying an ochre mutation on a multicopy plasmid (TA102), are added to the standard tester set.

Objective of the Study

The objective of this study was to determine the mutagenic potential of NITROGUANIDINE (LAIR Code TP 36A) by using the revised Ames Salmonella/Mammalian Microsome Mutagenicity Test.

MATERIALS AND METHODS

Test Compound

Chemical name: NITROGUANIDINE

Code number: LAIR Code No. TP 36A

Physical state: White crystalline solid

Source: Sunflower Army Ammunition Plant
De Soto, KS

Storage: NITROGUANIDINE was received from Sunflower Army Ammunition Plant, De Soto, KS, and assigned the LAIR Code number TP 36A. The test compound was stored at room temperature (21°C) until used.

Chemical Properties/Analysis: Data provided by Sunflower Army Ammunition Plant characterizing the chemical composition and purity of the test material are presented in Appendix A with confirmatory analysis of the test material performed by the Division of Toxicology, LAIR (Presidio of San Francisco, CA).

Test Solvent

The positive control chemicals were dissolved in grade I dimethyl sulfoxide (lot 113F-0450) obtained from Sigma Chemical Co. (St. Louis, MO). The test chemical was dissolved in the same lot of DMSO. Reagent grade water used in this assay is first passed through a Technic Series 300 Reverse Osmosis Unit (Seattle, WA), then through a Corning MP-1 Mega Pure System glass distillation unit (Corning Glass Works, Corning, NY) (4).

Chemical Preparation

On the day of dosing, the compound was dissolved directly into DMSO at a concentration of 28 mg/ml for the highest dose. Aliquots of this solution were used to prepare the serial dilutions.

Test Strains

Salmonella strains TA97, TA98, TA100, TA102, TA1535, TA1537, and TA1538 obtained from the laboratory of Dr. Bruce Ames, University of California, Berkeley, were used. These strains were maintained in our laboratory at -80°C. Quality control tests were run concurrently with the test substance to establish the validity of their special features and to determine the spontaneous reversion rate. Descriptions of the strains, their genetic markers, and the methods for strain validation are given in the LAIR SOP, OP-STX-1 (5).

Mammalian Microsome System

The S-9 (batch #R-315) was purchased from Microbiological Associates Inc. (Bethesda, MD). The optimal titer of this S-9, as determined by Microbiological Associates Inc., was 0.75 mg protein/plate.

Test Format

NITROGUANIDINE was evaluated for mutagenic potential according to the revised Ames method (3). A detailed description of the methodology is given in LAIR SOP, OP-STX-1 (5). The preincubation modification was chosen to enhance the sensitivity of the assay by exposing the bacteria to higher concentrations of test compound (and the activation products, when present) than would be possible in the standard plate incorporation assay. The bacteria were preincubated in the presence of the compound, both with and without metabolic activation, for 20 minutes on a shaker incubator at 37°C. A single preincubation tube was prepared for each top agar triplicate. Each preincubation tube contained 10 ml of a mixture which consisted of 1 ml of bacteria (16 hour culture), 1 ml of test compound (28 mg/ml or a serial dilution), 2 ml of S-9 if required, and the remaining volume nutrient broth. The highest dose (2.8 mg/ml) in the preincubation mixture approached the practical limits for nitroguanidine solubility in aqueous media (1). The top agar tubes were prepared by adding 0.7 ml from the preincubation tube to 2 ml of top agar. After mixing, the top agar was then overlaid on minimal glucose agar plates. These plates contained 2% glucose and Vogel Bonner "E" concentrate (6). Plates were incubated upside down in the dark at 37°C for 72 hours (Maron, 1985, personal communication). Plates were prepared in triplicate and the individual revertant counts were recorded. The average number of revertants at each dose level was compared to the average number of spontaneous revertants (negative control). The spontaneous reversion rate (with and without S-9) was

monitored by averaging the counts from two determinations run simultaneously with the test compound. The spontaneous reversion rate was determined by inoculating one set of plates before and one set after the test compound plates so that any change in spontaneous reversion rate during the dosing procedure would be detected. This spontaneous reversion rate was also compared with historical values for this laboratory and those cited in Maron and Ames (3). Sterility and strain verification controls were run concurrently. All reagents, test compounds, and media were checked for sterility by plating samples of each on MGA media and incubating them at 37°C with the test plates. The Salmonella strains were verified by a standard battery of tests. The integrity of the different Salmonella strains used in the assay was verified by the following standard tests:

-Lack of growth (inhibition) in the presence of crystal violet which indicated that the prerequisite alteration of the lipopolysaccharide layer of the cell wall was present.

-Growth in the presence of ampicillin-impregnated disks which indicated the presence of an ampicillin-resistant R Factor in all strains except TA1535, TA1537, and TA1538.

-Lack of growth (inhibition) following exposure to ultraviolet light which indicated the absence of the DNA excision-repair mechanism (for all strains except TA102).

Six known mutagens were tested as positive controls to confirm the responsiveness of the strains to the mutation process. Each strain must be tested with at least one positive control but may be tested with several. These compounds, benzo[a]pyrene (lot 18C-0378), 2-aminofluorene (lot 021547), 2-aminoanthracene (lot 020797), mitomycin-C (lot 015F-0655), 4-nitroquinoline-n-oxide (lot 89C-0710) and N-methyl-N'-nitro-N-nitrosoguanidine (lot 127C-0342), were obtained from Sigma Chemical Co. (St. Louis, MO). The test compound and mutagens were handled during this study in accordance with the standards published in NIH Guidelines for the Laboratory Use of Chemical Carcinogens (DHHS Publication No. (NIH) 81-2385, May 1981).

Data Interpretation

According to Brusick (7), a compound is considered mutagenic if a positive dose response (correlated dose

response) over three dose concentrations is achieved with at least the highest dose yielding a revertant colony count greater than or equal to twice the spontaneous colony count for the tester strains TA98 and TA100, or three times the spontaneous colony count for strains TA1535, TA1537, and TA1538 (3,5). A strong correlated dose response in strain TA100 without a doubling of the individual colony count may also be considered positive.

Maron and Ames (3) consider a compound mutagenic in tester strains TA97 and TA102 if a correlated dose response over three concentrations is achieved with the highest dose yielding a revertant colony count greater than or equal to twice the spontaneous colony count.

Deviations from the Protocol/SOP

The preincubation modification of the Ames Assay was chosen to enhance sensitivity by exposing bacteria to a higher concentration of compound, for a longer period of time. Volumes for the preincubation mixture were different from those specified in the SOP because of the limited solubility of nitroguanidine. This deviation has no impact on the validity of the study.

Storage of the Raw Data and Final Report

A copy of the final report, study protocols, raw data, SOPs, and an aliquot of the test compound will be retained in the LAIR archives.

RESULTS

Normal results were obtained for all sterility and strain verification tests during the Ames Test performed on 21-24 May, 1986 (Table 1). NITROGUANIDINE did not induce any appreciable increase in the revertant colony counts relative to those of the negative control cultures (Table 2).

A tabular presentation of raw data is included in Appendix B.

TABLE 1

STRAIN VERIFICATION AND STERILITY TESTING FOR
THE MUTAGENICITY DETERMINATION
ON NITROGUANIDINE (TP 36A)

GLP STUDY NUMBER 86008

STRAIN VERIFICATION

OBSERVATIONS*

STRAIN	HISTIDINE REQUIREMENT	AMPICILLIN RESISTANCE	UV REPAIR	CRYSTAL VIOLET	STERILITY CONTROL
TA97	NG	G	NG	NG	NG
TA98	NG	G	NG	NG	NG
TA100	NG	G	NG	NG	NG
TA102	NG	G	G	NG	NG
TA1535	NG	NG	NG	NG	NG
TA1537	NG	NG	NG	NG	NG
TA1538	NG	NG	NG	NG	NG

STERILITY CONTROL FOR MUTAGENICITY DETERMINATION

MATERIAL TESTED	OBSERVATION*
MINIMAL GLUCOSE AGAR PLATES	NG
TOP AGAR	NG
DILUENT WATER	NG
NUTRIENT BROTH	NG
TEST COMPOUND (HIGHEST DOSE)	NG
S-9	NG

*G = Growth, NG = No Growth

TABLE 2

NITROGUANIDINE

REVERTANTS/PLATE

MEAN ± 1SD

COMPOUND*	DOSE/PLATE	TA97	TA98	TA100	TA102
WITHOUT S-9					
NEG CONTROL	0.0 mg	88 ±16.1	14 ±3.3	111 ±10.2	173 ±13.2
MITO C	0.5 µg				1327 ±22.5
MNNG	2.0 µg			1460 ±149.1	
MNNG	20.0 µg				
NQNO	2.0 µg	252 ±92.2			
TP 36A	2.8 mg	66 ±10.6	19 ±0.6	88 ±22.5	119 ±1.7
TP 36A	1.4 mg	96 ±14.6	13 ±3.8	107 ±2.9	198 ±2.0
TP 36A	0.7 mg	83 ±15.3	12 ±4.6	106 ±11.0	166 ±24.4
TP 36A	0.35 mg	102 ±27.7	12 ±3.2	85 ±15.5	162 ±15.0
TP 36A	0.175 mg	96 ±7.6	20 ±2.0	96 ±--	146 ±35.6
TP 36A	0.0875 mg	93 ±6.1	16 ±5.5	99 ±13.1	172 ±12.7
WITH S-9					
NEG CONTROL	0.0 mg	110 ±16.6	35 ±10.8	125 ±21.7	282 ±5.4
2-AA	2.0 µg		298 ±73.4	563 ±86.5	
2-AF	2.0 µg	572 ±136.7	1683 ±42.5	706 ±86.5	
BP	2.0 µg	251 ±7.5	125 ±7.5	366 ±35.8	
TP 36A	2.8 mg	144 ±38.3	38 ±8.5	110 ±36.2	272 ±27.2
TP 36A	1.4 mg	107 ±9.5	25 ±2.1	122 ±26.6	282 ±20.4
TP 36A	0.7 mg	121 ±7.6	30 ±1.7	99 ±6.4	308 ±13.9
TP 36A	0.35 mg	101 ±9.0	26 ±1.0	127 ±22.7	280 ±10.2
TP 36A	0.175 mg	115 ±4.6	27 ±0.6	105 ±--	267 ±59.5
TP 36A	0.0875 mg	107 ±6.6	33 ±4.5	129 ±9.7	298 ±15.1

*MITO-C=mitomycin-C, MNNG=N-methyl-N'-nitro-N-nitrosoguanidine, NQNO=4-nitroquinoline-n-oxide, AA=2-aminoanthracene, AF=2-aminofluorene, BP=benzo[a]pyrene.

TABLE 2, (continued)

NITROGUANIDINE

REVERTANTS/PLATE

MEAN ± 1SD

COMPOUND*	DOSE/PLATE	TA1535	TA1537	TA1538
WITHOUT S-9				
NEG CONTROL	0.0 mg	11 ±3.0	10 ±3.2	18 ±6.4
MNNG	20.0 µg	2224 ±378.2		
TP 36A	2.8 mg	21 ±2.5	19 ±4.6	19 ±4.0
TP 36A	1.4 mg	13 ±4.7	7 ±0.6	10 ±4.6
TP 36A	0.7 mg	10 ±2.3	12 ±5.6	9 ±3.6
TP 36A	0.35 mg	10 ±1.2	10 ±0.0	16 ±5.9
TP 36A	0.175 mg	14 ±4 0	6 ±2.0	13 ±0.6
TP 36A	0.0875 mg	16 ±6.4	5 ±0.6	17 ±1.0
WITH S-9				
NEG CONTROL	0.0 mg	11 ±1.7	12 ±4.1	27 ±2.4
2-AA	2.0 µg		318 ±55.2	572 ±231.1
2-AF	2.0 µg			1168 ±173.9
BP	2.0 µg		48 ±5.9	74 ±39.0
TP 36A	2.8 mg	9 ±3.6	10 ±3.6	25 ±9.6
TP 36A	1.4 mg	18 ±13.0	10 ±4.2	34 ±1.7
TP 36A	0.7 mg	12 ±4.9	9 ±2.5	25 ±4.9
TP 36A	0.35 mg	14 ±4.5	11 ±4.2	14 ±6.5
TP 36A	0.175 mg	15 ±3.0	12 ±4.6	24 ±7.0
TP 36A	0.0875 mg	16 ±11.8	12 ±2.5	17 ±1.0

*MITO-C=mitomycin-C, MNNG=N-methyl-N'-nitro-N-nitrosoguanidine, NQNO=4-nitroquinoline-n-oxide, AA=2-aminoanthracene, AF=2-aminofluorene, BP=benzo[a]pyrene.

DISCUSSION

Certain test criteria must be satisfied before an Ames test can be considered a valid assessment of a compound's mutagenic potential. First, the special features of the Ames strains must be verified. These features include demonstration of ampicillin resistance, alterations in the lipopolysaccharide layer, and deficiency in DNA excision-repair (except TA102). Second, the Salmonella strains must be susceptible to mutation by known mutagens. Third, the optimal concentration of the test compound must be determined by treating TA100 with a broad range of doses and observing the potential toxic effects on formation of macrocolonies and microcolonies. If these tests are performed and expected data are obtained, then the results of an Ames test can be considered valid.

After validation of bacterial strains and selection of optimal sublethal doses, NITROGUANIDINE was evaluated in the Ames preincubation test. Criteria for a positive response include both a correlated dose response over three dose concentrations, and a revertant colony count at least two times for TA97, TA98, TA100, and TA102 (2,7) or three times for TA1535, TA1537, and TA1538 (3,5) the spontaneous revertant colony count. NITROGUANIDINE did not induce the requisite dose-response relationship or the increase in revertant colony counts necessary for a positive response. Thus, the results of this test indicate that NITROGUANIDINE is not mutagenic when evaluated in the Ames test.

CONCLUSION

NITROGUANIDINE was evaluated for mutagenic potential in the Ames test, both in the presence and absence of metabolic activation, and did not induce a positive mutagenic response under conditions of this study.

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APPENDIX A

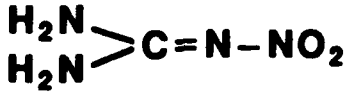
CHEMICAL DATA

Chemical name: Nitroguanidine (NGu)

Other listed names: Guanidine, Nitro; alpha-Nitroguanidine; beta-Nitroguanidine

LAIR Code: TP 36A

Structural formula:



Molecular formula: CH4N402

Molecular weight: 104.1

pH range of dosing suspensions: 6.7 - 7.4(1)

Physical state: White Powder

Melting point: 232° C(2)

Source: Hercules Aerospace Division
Sunflower Ammunition Plant
DeSoto, Kansas

Lot No. SOW84K010-A-001

Analytical data/purity:
The major peaks in the infrared spectrum of the compound were observed at 3450, 3396, 3342, 3278, 3201, 1666, 1634, 1525, 1404, 1314, 1151, 1045, 782 cm-1.(3) The spectrum obtained for the test compound in our lab was identical to the Sadtler standard spectrum for nitroguanidine.(4) HPLC showed only one peak (retention time 4.9 min).(5) The conditions employed were as follows: column, Brownlee RP-18 (4.6 x 250 mm); solvent, 10% methanol-90% water; flow rate, 0.7 ml/min; oven temperature, 50°C; monitoring wavelength, 265 nm.

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APPENDIX B-1
RAW DATA TABLE
NEGATIVE CONTROL
NITROGUANIDINE (TP 36A)

COMPOUND	DOSE	TA97	TA98	TA100	TA102	TA1535	TA1537	TA1538
WITHOUT S-9								
NEG CONTROL (START RUN)	0.0 mg/plate	101	14	105	178	16	11	21
		79	12	107	193	8	14	24
		61	10	97	174	11	13	26
NEG CONTROL (END RUN)	0.0 mg/plate	100	12	115	158	12	8	13
		101	16	126	159	10	6	10
		85	19	117	178	8	8	15
WITH S-9								
NEG CONTROL (START RUN)	0.0 mg/plate	104	45	134	283	13	10	26
		92	36	116	275	9	18	29
		100	46	99	280	13	6	27
NEG CONTROL (END RUN)	0.0 mg/plate	140	36	149	288	12	15	28
		113	29	*	*	10	12	22
		113	17	*	*	10	11	27

*plate contaminated

APPENDIX B-2
RAW DATA TABLE
POSITIVE CONTROLS

COMPOUND*	DOSE	TA97	TA98	TA100	TA102	TA1535	TA1537	TA1538
2-AA	2.0 µg/plate		377	565			376	566
			285	476			266	806
			232	649			313	344
2-AF	2.0 µg/plate	730	1683	682				1235
		501	1641	634				1299
		486	1726	802				971
BP	2.0 µg/plate	244	118	335			46	43
		259	133	405			55	62
		251	125	357			44	118
MITO C	0.5 µg/plate				1341			
					1301			
					1339			
MNNG	2.0 µg/plate			1601				
				1304				
				1475				
MNNG	20.0 µg/plate					1801		
						2340		
						2530		
NQNO	2.0 µg/plate	289						
		147						
		320						

*MITO-C=mitomycin-C, MNNG=N-methyl-N'-nitro-N-nitrosoguanidine, NQNO=4-nitroquinoline-n-oxide, AA=2-aminoanthracene, AF=2-aminofluorene, BP=benzo[a]pyrene.

APPENDIX B-3

RAW DATA TABLE

NITROGUANIDINE (TP 36A)

WITHOUT S-9

COMPOUND	DOSE	TA97	TA98	TA100	TA102	TA1535	TA1537	TA1538
TP 36A	2.8 mg/p	68	19	80	118	21	24	23
		55	18	70	121	24	15	20
		76	19	113	118	19	18	15
TP 36A	1.4 mg/p	84	15	109	198	8	7	15
		112	9	109	196	15	6	9
		91	16	104	200	17	7	6
TP 36A	0.7 mg/p	89	15	93	139	7	17	10
		95	7	113	174	11	13	5
		66	15	111	186	11	6	12
TP 36A	0.35 mg/p	133	10	100	177	11	10	18
		95	16	86	147	9	10	9
		79	11	69	162	11	10	20
TP 36A	0.175 mg/p	87	22	96	180	16	8	13
		101	20	*	150	9	4	14
		99	18	*	109	16	6	13
TP 36A	0.0875 mg/p	100	16	111	186	9	5	16
		90	22	85	161	21	5	17
		89	11	100	170	19	4	18

*plate contaminated

APPENDIX B-4

RAW DATA TABLE

NITROGUANIDINE (TP 36A)

WITH S-9

COMPOUND	DOSE	TA97	TA98	TA100	TA102	TA1535	TA1537	TA1538
TP 36A	2.8 mg/p	103	37	92	303	8	11	27
		179	30	152	254	13	13	15
		149	47	87	258	6	6	34
TP 36A	1.4 mg/p	110	27	106	259	10	9	36
		96	24	108	297	11	7	33
		114	23	153	291	33	15	33
TP 36A	0.7 mg/p	114	31	94	300	8	9	23
		129	28	*	324	15	11	22
		119	31	103	300	*	6	31
TP 36A	0.35 mg/p	95	27	119	287	10	10	14
		96	25	153	268	19	8	7
		111	26	110	284	14	16	20
TP 36A	0.175 mg/p	120	27	105	200	12	17	19
		114	28	*	313	18	9	21
		111	27	*	289	16	9	32
TP 36A	0.0875 mg/p	100	29	118	296	9	15	18
		108	33	131	284	10	12	17
		113	38	137	314	30	10	16

*plate lost

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